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Molecular Cloning and Characterization of the *STE7* and *STE11* Genes of *Saccharomyces cerevisiae*

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In the yeast *Saccharomyces cerevisiae*, haploid cells occur in one of the two cell types, a or α. The allele present at the mating type (*MAT*) locus plays a prominent role in the control of cell type expression. An important consequence of the elaboration of cell type is the ability of cells of one mating type to conjugate with cells of the opposite mating type, resulting in yet a third cell type, an *a*/α diploid. Numerous genes that are involved in the expression of cell type and the conjugation process have been identified by standard genetic techniques. Molecular analysis has shown that expression of several of these genes is subject to control on the transcriptional level by the *MAT* locus. Two genes, *STE7* and *STE11*, are required for mating in both haploid cell types; *ste7* and *ste11* mutants are sterile. We report here the molecular cloning of *STE7* and *STE11* genes and show that expression of these genes is not regulated transcriptionally by the *MAT* locus. We also have genetically mapped the *STE11* gene to chromosome XII, 40 centimorgans from *ura4*.

Haploid cells of the yeast *Saccharomyces cerevisiae* exist as one of the two cell types, **a** or α. Expression of cell (or mating) type is controlled by the allele *MAT***a** or *MAT*α, present at the mating type (*MAT*) locus (4, 22). Thus, with the exception of the *MAT* locus, all haploids contain the genetic information for both cell types, but only *MAT*α cells express α-specific genes, whereas **a**-specific genes are expressed only in *MAT***a** cells. This selective activation of **a**- and α-specific genes by the *MAT* locus occurs on the transcriptional level (2, 27, 30). In *MAT*α cells, all α-specific genes are positively regulated by one of the *MAT*α-encoded genes, *MAT*α*1*. The other *MAT*α gene, *MAT*α*2*, negatively controls **a**-specific genes (32, 36). The **a**-specific genes are expressed constitutively in *MAT***a** cells.

An important consequence of the elaboration of cell type in yeast haploids is the ability of cells of one mating type to mate with cells of the opposite mating type, resulting in yet a third cell type, the **a**/α diploid. These diploids do not express either of the haploid-specific cell types and are mating incompetent. This negative regulation of **a**- and α-specific genes is believed to be mediated by the combined interaction of the *MAT*α*2* and *MAT***a***1* gene products (7, 10, 13, 21, 27, 30, 32).

The conjugation reaction is triggered when cells of one mating type recognize the cell-type-specific mating hormone secreted by cells of the opposite mating type. This recognition event probably involves cell-type-specific hormone receptors (6). Two of the events that occur after hormone exposure are cell aggregation and the arrest of each cell at start in the G1 phase of the cell division cycle. These events, which are followed by cell fusion and karyogamy, culminate in zygote formation, from which **a**/α diploid buds emerge.

Numerous genes that are required for conjugation have been identified. Because mating competence requires the expression of a haploid cell type, mutation of many (but not all) **a**- and α-specific genes causes sterility (3, 12, 14, 15, 17, 29, 31). Cell-type-specific sterility can also result from mutation of a gene whose expression is not mating-type dependent. For example, mutant alleles of the α-specific sterile gene *STE13* confer sterility only in *MAT*α haploids, although the gene is expressed in all cell types (30). A second class of sterile genes has been called nonspecific because mutations at these loci cause sterility in cells of both mating types (3, 14, 15). Two members of this latter class, *STE4* and *STE5*, have been implicated in hormone-mediated cell division cycle arrest (26), but very little is known about the role(s) of other nonspecific sterile genes (*STE7*, *STE11*, *STE12*) in the mating process.

To elucidate the function(s) of the *STE7* and *STE11* genes in conjugation on a molecular level, we have isolated and characterized plasmid clones carrying the wild-type alleles of these genes. Furthermore, we have used these clones as substrates for the construction of *STE7* and *STE11* deletions in vitro, which were then integrated into the *S. cerevisiae* genome, replacing the corresponding wild-type *STE* allele in vivo. With these tools, the *STE7* and *STE11* transcripts were identified, and the transcriptional properties of these two genes were assessed. The results of these experiments demonstrate that transcription of *STE7* and *STE11* is not controlled by the *MAT* locus and that these genes encode nonessential functions. We also report the genetic mapping of the *STE11* gene.

MATERIALS AND METHODS

Strains and genetic procedures. The strains and plasmids used in this study are listed in Tables 1 and 2, respectively. The *ste7* and *ste11* mutations were isolated by Hartwell (3) and confer a temperature-sensitive conjugation-deficient phenotype in both *MAT***a** and *MAT*α haploids. The *ste11* deletion strains DC24 through DC26 are isogenic to strain EG123. Strains DC60 and DC64 harbor the *ste7*-Δ*1* and *ste7*-Δ*2* deletions, respectively, and are isogenic to strain A2α, whereas strains DC52 (*ste7*-Δ*1*) and DC56 (*ste7*-Δ*2*) are isogenic to strain 80. These deletion strains were constructed by cotransforming the wild-type parent strain with a deletion-bearing DNA fragment and either YRp7 or YEp13. Trp^+ (or Leu^+) transformants were screened for mating competence. Strain DC39 is a *MAT*α *ste11*-Δ*1* segregant of a tetrad derived from strain DC26. The *mat* mutations were

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TABLE 1. Strains

Strain	Haploid parents	Genotype[a]	Source or comments
Haploids			
A2α		α *leu2-2 leu2-112 his3-11 his3-15 can1*	V. MacKay
XCO26C		α *ste11*(Ts) *leu2-2 leu2-112 his3-11 his3-15 his4-580 trp1*	V. MacKay
XCO14D		a *ste7*(Ts) *leu2-2 leu2-112 trp1 can1 ade2 adeX*	V. MacKay
DCX105-27A		a *ste11*(Ts) *spo11 leu2-2 leu2-112 trp1 cyh2 His*$^-$	This work
K381-9D		α *spo11 ura3 ade6 arg4 aro7 asp5 lys2 met14 pet17 trp1*	C Waddell
K382-23A		a *spo11 ura3 can1 cyh2 ade2 his7 hom3*	C. Waddell
Be287		α *car2 ura4 gal2*	T. Petes
AH229T9		a *leu2-2 leu2-113 RDN1::LEU2 can1 gal2 his4*	T. Petes
DC11		α *ste7*(Ts) *leu2-2 leu2-112 his3-11 his3-15 can1 trp1* Ade$^-$	This work
DC14		a *leu2-2 leu2-112 ade2-1*	This work
DC15		a *leu2-2 leu2-112 adeX lys2*	This work
DC23		a *ste11*(Ts) *leu2-2 leu2-112 trp1*	This work
DC24		a *ste11-Δ1 leu2 his4-519 trp1 ura3 can1-101*	This work
DC25		a *ste11-Δ1 leu2 his4-519 trp1 ura3 can1-101*	This work
DC27		a *ste11*(Ts)::pSTE11.2 *his3-11 his3-15 his4-580 leu2-2 leu2-112 trp1*	Integrative transformant of XCO26C
DC28		α *ste7*(Ts)::pSTE7.2 *leu2-2 leu2-112 his3-11 his3-15 can1 trp1* Ade$^-$	Integrative transformant of DC11
DC39		α *ste11-Δ1 leu2 his4-519 trp1 ura3 can1-101*	This work
DC52		a *ste7-Δ1 leu2 lys2 trp1*	This work
DC56		a *ste7-Δ2 leu2 lys2 trp1*	This work
DC60		α *ste7-Δ1 leu2-2 leu2-112 his3-11 his3-15 can1*	This work
DC64		α *ste7-Δ2 leu2-2 leu2-112 his3-11 his3-15 can*	This work
DC99		α *ste11*(Ts) *leu2-2 leu2-112 suc2-Δ9 ura3*	This work
EG123		a *leu2 his4-519 trp1 ura3 can1-101*	This work
80		a *leu2 lys2 trp1*	K. Tatchell
53		*matα2-182 leu2 his4-519 trp1 ura3 can1-101*	K. Tatchell
27		*matα2-75 leu2 his4-519 trp1 ura3 can1-101*	K. Tatchell
6		*matα1-189 matα2-75 leu2 his4-519 ura3 can1-101*	K. Tatchell
22		*matα1-113 leu2 his4-519 trp1 ura3 can1-101*	K. Tatchell
Diploids			
DC26		a *leu his4-519 trp1 ura3 can1-101 ste11-Δ1* / α *leu2 his4-519 trp1 ura3 can1-101* +	This work
DCX33	DC14 / DC27	a *leu2-2 leu2-112* + *ade2-1* + + + / α *leu2-2 leu2-112 his4-580* + *his3-11 his3-15 trp1 ste11*(Ts)::pSTE11.2	This work
DCX45	EG123 / A2α	a *leu2 his4-519 trp1 ura3 can1-101* + / α *leu2-2 leu2-112* + + + *can1 his3-11 his3-15*	This work
DCX47	23ax50 / A2α	*mat1-50 leu2 his4-519 trp1 ura3 can1-101* + / α *leu2-2 leu2-112* + + + *can1 his3-11 his3-15*	This work
12		*MATα1 matα2-182 leu2 his4-519* + *trp1 ura3 can1-101* / *MATa leu2* + *lys2 trp1* + +	K. Tatchell
DCX59	DC15 / DC28	a *leu2-2 leu2-112* + + *lys2 adeX* + + / α *leu2-2 leu2-112 ste7*(Ts)::pSTE7.2 *trp1* + Ade$^-$ *his3-11 his3-15 can1*	This work
DCX121	DCX105-27A / K381-9D	a *leu2-2 leu2-112 spo11 ste11*(Ts) + + + + + + + *trp1 cyh2* His$^-$ / α + + *spo11* + *asp5 ade6 arg4 aro7 lys2 met14 pet17 trp1* + +	This work
DCX130	AH229T9 / DC99	a *leu2-2 leu2-112 his4* + *gal2 RDN1::LEU2* + + *can1* / α *leu2-2 leu2-112* + *suc2-Δ9* + + *ste11*(Ts) *ura3* +	This work
DCX131	Be287 / DC99	a + *gal2 ura4 car2* + + + / α *leu2-2 leu2-112* + + + *ste11*(Ts) *suc2-Δ9 ura3*	This work

[a] *adeX*, *X* is not 1, 2, or 6.

generated by in vitro mutagenesis and have been described elsewhere (33). These mutant alleles were introduced into the *S. cerevisiae* genome as described above.

Complete yeast extract-peptone-dextrose (YEPD), yeast extract-peptone-glycerol, minimal (SD), synthetic complete, and sporulation media were prepared as described by Sherman et al. (25), except that synthetic complete medium lacked aspartic acid, glutamic acid, serine, and valine. Omission media (e.g., leucine omission) contained all of the components of synthetic complete medium except the one

TABLE 2. Plasmids

Plasmid	Description
pSTE7.1	Original 2.8-kb *STE7* clone in YEp13
pSTE7.2	2.8-kb *STE7* clone in YRp7
pSTE7.3	2.8-kb *STE7* clone in pBR322
pSTE11.1	Original 5.6-kb *STE11* clone in YEp13
pSTE11.2	5.6-kb *STE11* clone in YRp7
pSTE11.3	5.6-kb *STE11* clone in pBR322

indicated. All solid media contained 2% agar. Cycloheximide plates were prepared as described by Klapholz and Esposito (9). Canavanine was added to arginine omission medium to a final concentration of 60 μg/ml. Media containing 1 mg of ornithine per ml as the sole nitrogen source were used to score *car2* mutants.

Bacterial media were prepared as described by Miller (18). Luria broth and plates were supplemented with ampicillin (50 μg/ml) or tetracycline (25 μg/ml). These media were solidified with 1.5% agar.

Genetic crosses and tetrad analysis were performed by standard techniques (26). Diploids were isolated by prototroph selection. Matings involving temperature-sensitive *ste* strains were carried out at room temperature; all other matings were performed at 30°C. Single diploid colonies were transferred to sporulation medium by replica plating. The Ste phenotype was determined by assaying the ability of a strain to mate and form prototrophic diploids with one of two test strains. Strains harboring a temperature-sensitive *ste* mutation are capable of mating at 22 but not at 33°C (3). Sterility is temperature independent in the *ste* deletion strains.

All yeast transformations were performed as described by Beggs (1). In most cases, individual transformants were picked from the original transformation plates and used directly. In other cases, such as the original isolation of plasmids containing the *STE7* and *STE11* genes or the identification of integrative transformants containing the *ste7* or *ste11* deletions, all of the transformants were recovered from the top agar by passage through an 18-gauge needle into 10 ml of the appropriate medium. The transformants were then plated at an appropriate density (150 or 1,000 colonies per plate) and screened for the desired phenotype.

Plasmid stability tests. Individual colonies from putative Ste^+ transformants were transferred to a nonselective complete medium, grown overnight at 30°C, and then streaked. The resultant colonies were tested for their Leu phenotype by replica plating to leucine omission medium and for their Ste phenotype by mating to the appropriate test strain on rich medium at the restrictive temperature.

Genetic mapping of *STE11*. The chromosomal location of *STE11* was determined by the method of Klapholz and Esposito (9). This technique is based on the observation that *spo11* homozygotes fail to undergo meiotic recombination. Thus, genes that reside on the same chromosome will exhibit strong linkage in *spo11* diploids regardless of the actual genetic distance that separates them. DCX105-27A, a *ste11 spo11* ascosporal segregant from a cross between strains XCO26C and K382-23A, was crossed to the *spo11* mapping strain K381-9D. In the *spo11* homozygote DCX121, the temperature-sensitive *ste11* allele is in repulsion to genes that uniquely mark a number of chromosomes. Physical linkage of *ste11* to the chromosome bearing a given marker gene is indicated by the failure to detect in the resultant ascosporal segregants a recombinant carrying a double mutation. Cycloheximide-resistant meiotic progeny of this cross strain, DCX121, were isolated and analyzed as described by Klapholz and Esposito (9). After the *STE11* gene was localized to a chromosome, its precise map location was determined by standard genetic means.

Biochemical procedures. Genomic *S. cerevisiae* DNA was isolated as described by Winston et al. (37). Highly purified plasmid DNA was isolated by the alkaline lysis method described by Maniatis et al. (16). A rapid plasmid preparation procedure was also used (24).

Total *S. cerevisiae* RNA was isolated by a modified version of the method of Sprague et al. (30). Cells were grown to mid-log phase in 100 ml of complete (YEPD) broth (A_{600} = 2), harvested by centrifugation, washed once in sterile distilled water, and suspended in 3 ml of chilled RNA buffer (50 mM Tris-hydrochloride, pH 7.4, 100 mM NaCl, 10 mM EDTA, 0.01% diethylpyrocarbonate). An equal volume of glass beads was added to the cell suspension, and the cold mixture was vortexed six times for 15 s with 45 s of chilling between mixings. RNA buffer (3 ml) and sodium lauryl sulfate to 1.0% (wt/vol) were added to the crude lysates, after which 6 ml of RNA buffer-saturated phenol was added. After thorough mixing, the aqueous phase was recovered after centrifugation. The phenol extraction step was repeated twice, followed by two extractions with a mixture of chloroform and isoamyl alcohol (24:1). Nucleic acid was precipitated from the solution with 2 volumes of ethanol at −70°C, and the precipitate was dissolved in 10 mM Tris-hydrochloride (pH 8.0) containing 1 mM EDTA and 0.1% sodium lauryl sulfate. After incubation for 3 min at 70°C, NaCl was added to 0.5 M. The RNA sample was applied to an oligodeoxythymidylate-cellulose (P-L Biochemicals, Inc.) column that had been equilibrated with the same buffer without sodium lauryl sulfate. After being loaded, the column was washed once with equilibration buffer and once with a buffer containing 10 mM Tris-hydrochloride (pH 8.0), 1 mM EDTA, and 0.25 M NaCl. Polyadenylated [$poly(A)^+$] RNA was eluted with 10 mM Tris-hydrochloride (pH 8.0)–1 mM EDTA. The eluate was then concentrated by ethanol precipitation.

Restriction endonucleases were purchased from either Bethesda Research Laboratories, Inc., or New England BioLabs, Inc. DNA polymerase was obtained from Bethesda Research Laboratories, and T4 DNA ligase was from New England BioLabs. All enzymes were used as specified by the manufacturer.

Nucleic acid hybridization of *S. cerevisiae* DNA was performed by the method of Southern (28). $Poly(A)^+$-selected, glyoxylated RNA was examined by Northern blot hybridization analysis (34). ^{32}P-labeled probes were prepared by nick translation (23). Autoradiography was performed at −70°C with Kodak XAR5 film and Du Pont Lightning-Plus intensifying screens. ^{32}P-labeled probes were removed from the filters by placing the moistened filters in 95°C water and allowing the mixture to cool to room temperature (27). The regenerated filters were then prehybridized and hybridized as previously described (34).

RESULTS AND DISCUSSION

Isolation of *STE7* and *STE11* plasmid clones. Plasmid DNA from a bank of *Sau*3A partially digested genomic *S. cerevisiae* DNA contained in the *Escherichia coli-S. cerevisiae* shuttle vector YEp13 (20) was introduced by transformation into the temperature-sensitive strains XCO14D (*ste7*) and XCO26C (*ste11*). At least 15,000 Leu^+ transformants were recovered from each experiment and screened for mating

TABLE 3. Frequency of recombinants carrying double mutations

Gene assayed	Chromosome marked	No. of Ste11$^-$ X$^-$[a] segregants	No. of Cyh2$^-$ segregants scored
ade6	VII	4	164
arg4	VIII	42	164
aro7	XVI	47	164
asp5	XII	0	164
lys2	II	49	164
met14	XI	33	133
pet17	XV	58	164
ura3	V	10	50

[a] X$^-$ represents the mutant phenotype of the marker gene assayed (e.g., Ade6$^-$, Arg4$^-$, etc.).

competence at the restrictive temperature (33°C). One Leu$^+$ XCO26C and two Leu$^+$ XCO14D transformants proved to be fertile (Ste$^+$). That their Ste$^+$ phenotype was due to the presence of a plasmid containing the appropriate *STE* gene was determined from a number of genetic tests. To ensure that the Ste$^+$ phenotype was not a consequence of a mutational or reversion event, cosegregation of the Ste$^+$ and plasmid-associated (Leu$^+$) phenotypes was assayed. Because plasmids in *S. cerevisiae* are not distributed equally among mother and daughter cells during mitotic growth, daughter cells that do not receive the plasmid will arise frequently on nonselective media and are identified by their Leu$^-$ phenotype. From each of the original Leu$^+$ transformants isolated, Leu$^-$ Ste$^-$ segregants were frequently recovered, indicating that the Ste$^+$ phenotype is plasmid borne. That plasmid DNA isolated from the transformants complemented the appropriate *ste* defect when reintroduced into the temperature-sensitive *ste* strains provided additional physical evidence that the Ste$^+$ phenotype is plasmid associated. However, no cross-complementation was detected, i.e., the putative *STE7* gene did not confer fertility on *ste11* mutants and vice versa.

Genetic evidence that the plasmids harbored the wild-type *STE* allele rather than a suppressor was obtained by mapping the cloned *STE* DNA in transformants in which the *STE*-containing plasmids had integrated into the chromosomal region homologous to the cloned insert. Because introduction of transforming DNA into the *S. cerevisiae* genome occurs by homologous recombination (5), our demonstration that the *STE*-bearing plasmids had integrated at the relevant *STE* locus indicated that these two regions shared DNA sequence homology and thus that the cloned yeast DNA carried the correct *STE* gene. The cloned inserts of pSTE7.1 and pSTE11.1 were subcloned into YRp7, generating plasmids pSTE7.2 and pSTE11.2, respectively (Table 2). Unlike YEp13, the YRp7 vector carries the selectable yeast marker *TRP1* and can be stably integrated and maintained in the *S. cerevisiae* genome. Integration of each plasmid was directed into the chromosomal region with homology to the cloned insert by cleaving the plasmid at a restriction site in or close to the putative *STE* gene (22). Plasmid pSTE7.2 was digested with *Kpn*I; pSTE11.2 was digested with *Xho*I. These linear molecules were then used to transform strains XCO26C [*ste11*(Ts)] and DC11 [*ste7*(Ts)], respectively, to tryptophan protrophy. A stable Trp$^+$ Ste$^+$ transformant of each recipient strain was isolated and analyzed genetically as follows. First, each transformant was crossed to a *trp1 ste7* (or *ste11*) haploid. The 2:2 segregation pattern for both *trp1* and *ste7* (or *ste11*) in the resultant tetrads indicated that integration had occurred at only one genomic site. In addition, as expected, *TRP1* and *STE7(11)* were linked; no tetratype asci (i.e., recombinant spores) were detected in the 10 to 20 tetrads analyzed per cross.

In the second set of crosses, each integrative transformant was crossed to a *TRP1 STE7(11)* strain. If the integration of the wild-type, plasmid-borne *STE* gene occurred at the *STE11* locus, then the *STE11* and *ste11*(Ts) alleles would be closely linked and no Ste$^-$ meiotic progeny would be obtained. However, recovery of the recessive allele from these crosses would indicate that the integration event had not occurred at the segregating locus and that the putative *STE* clone carried a suppressor. Tetrads derived from strain DCX33 displayed only 4 Ste$^+$:0 Ste$^-$ segregation for *STE11* and 4 Trp$^+$:0 Trp$^-$, 3 Trp$^+$:1 Trp$^-$, and 2 Trp$^+$:2 Trp$^-$ segregation for *TRP1*. These results provide strong evidence that pSTE11.2 integrated at or near the *STE11* locus. Therefore, we concluded that pSTE11.1 and pSTE11.2 contain the wild-type allele of *STE11*. The results of the analogous cross strain, DCX59, with the pSTE7.2 integrative transformant paralleled those of strain DCX33. No Ste$^-$ segregants were observed in 17 tetrads, yet Trp$^-$ ascospores were frequently detected. Thus, the *STE7* gene is contained in pSTE7.1 and pSTE7.2.

Genetic mapping of *STE11*. The chromosomal location of the *STE11* gene was determined from a combination of meiotic mapping methods. First, this gene was assigned to a chromosome by using the *spo11* mapping method of Klapholz and Esposito (9) as described above.

Cycloheximide-resistant meiotic progeny of strain DCX121 were selected and analyzed (Table 3). Of the 121 Ste$^-$ segregants examined, none were Asp$^-$, indicating that *ste11* is located on chromosome XII. In contrast, Ste$^-$ double mutants were frequently observed for all of the other markers segregating in the cross. (The apparently low number of Ade$^-$ segregants was a consequence of selecting *cyh2* ascosporal segregants. Since the *cyh2* chromosome contains the *ADE6* allele, Ade$^-$ spores should, and did, arise infrequently. Of the six Ade$^-$ segregants recovered, four were sterile, indicating that *ste11* is not on chromosome VII.) Examination of the strain DCX121 segregants also revealed a peculiar transmission of the *ste11*-bearing chromosome: 121 of the 164 Cyh2$^-$ segregants analyzed were Ste$^-$. A similarly skewed transmission of *asp5* was also observed: only six Asp$^-$ spores were recovered. Because the *ste11* mutation is in repulsion with the *asp5* mutation, the qualitative reciprocity in the recovery of these mutant alleles provides further evidence that *ste11* resides on chromosome XII. None of the other markers segregating in the cross exhibited this peculiar pattern of transmission (Table 3).

TABLE 4. Tetrad analysis of *STE11* crosses

Strain	Gene pair	No. of tetrads			Distance (centimorgans)
		Parental ditype	Nonparental ditype	Tetratype	
DCX131	*ura4-car2*	31	0	46	29.9
	ura4-ste11	24	2	51	40.9
	car-ste11	18	8	47	>50
DCX130[a]	*leu2-ste11*	11	7	29	>50
	leu2-his4	4	15	30	>50

[a] The *LEU2* allele is located in the *RDN1* locus.

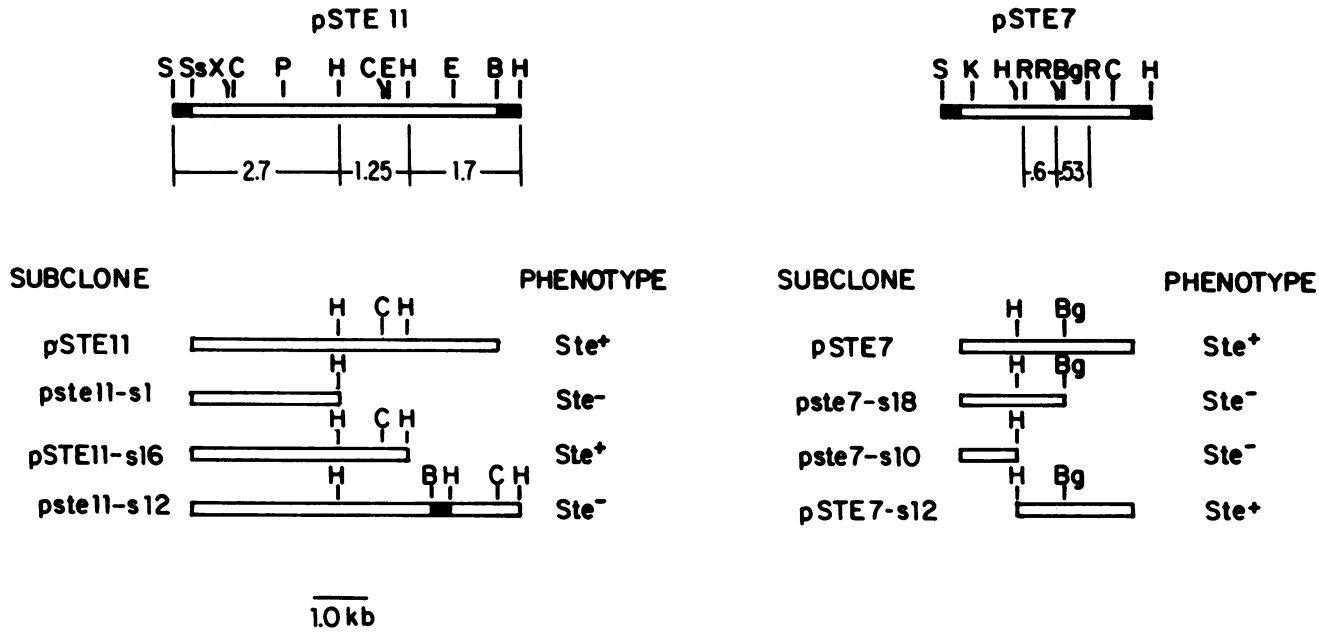

FIG. 1. Restriction maps and subclone analyses of pSTE7 and pSTE11. Open bars, Cloned *S. cerevisiae* insert DNA; solid bars, plasmid sequences. The structures and relevant restriction sites of recombinant plasmid subclones are shown below each map. The Ste phenotype of the *S. cerevisiae ste7*(Ts) and *ste11*(Ts) recipient strains transformed with each subclone is shown at the right of each subclone. Restriction endonuclease sites: B, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; R, *Eco*RV; S, *Sal*I; Ss, *Sst*I; X, *Xho*I.

Proof that *ste11* is located on chromosome XII was derived from tetrad analysis of strain DCX131. *ste11* was unlinked to *car2* and loosely linked (40.9 centimorgans) to *ura4* (Table 4). The *car2-ura4* map distance obtained for this cross was 29.9 centimorgans, which was in good agreement with previously published results (19). These data suggest the gene order *ste11-ura4-car2*. Three-factor analysis of strain DCX131 also supported this gene order, as *ste11*, *ura4*, *CAR2*, and *STE11 URA4 car2* recombinant ascospores were recovered less frequently than the other recombinant classes (data not shown). In strain DCX130, no linkage was detected between *ste11* and the centromere-proximal gene *RDN1* (marked by *LEU2*; Table 4). Therefore, the order of *ste11*, *ura4*, and *car2* relative to the centromere could not be determined.

These mapping studies demonstrate that *STE11* defines a new site on the genetic map. Similarly, *STE7* resides on the left arm of chromosome IV, between *cdc9* and *cdc36* (19). Because both of these genes mapped to unique chromosomal positions rather than to sites defined by previously identified genes, these mapping data do not shed any insight into the function(s) that *STE7* and *STE11* encode.

In vitro construction of *STE7* and *STE11* deletions. Identification of the *STE*-encoded transcripts was necessary to assess the transcriptional properties of the *STE7* and *STE11* genes as a function of the genetic constitution of the *MAT* locus. Since temperature-sensitive mutant alleles of these genes were expected to be transcriptionally active and to produce a normal mRNA, it seemed unlikely that analysis of the temperature-sensitive *ste* strains would permit positive identification of the relevant *STE* products. However, *ste* deletion-bearing strains should produce either an aberrant RNA or no RNA at all, permitting clear identification of the *STE* mRNA. To construct such deletion mutants, the *STE7*- and *STE11*-bearing clones were subcloned and analyzed to identify subfragments carrying the *STE* gene. This information was used to construct partial deletions of these genes that could, by integrative transformation, be used to replace the corresponding wild-type *STE* sequences in vivo.

A partial restriction endonuclease map of the pSTE7.1 and pSTE11.1 inserts is shown in Fig. 1. To further localize the *STE7* gene on the clone, various restriction fragments were subcloned and assayed by yeast transformation for the ability to restore fertility to an *ste7*(Ts) strain. Of the several subclones tested (Fig. 1), only plasmid pSTE7-s12, containing the 2.3-kilobase (kb) *Hin*dIII fragment, complemented the Ste$^-$ phenotype of the mutant haploids.

A slightly different approach was employed in the analogous analysis of pSTE11.1. This plasmid was digested to completion with *Hin*dIII and religated. The resultant rearranged plasmids were tested for the ability to complement the *ste11* defect. The structures of the recovered molecules are shown in Fig. 1, as are the results of this subclone analysis. Simple recircularization of the digested plasmid resulted in a plasmid, represented by p*ste11*-s1, which contained 2.7 kb of the original insert and was insufficient to confer an Ste$^+$ phenotype on the *ste11*(Ts) recipient. Recombinant plasmids that contained this 2.7-kb fragment and the internal 1.25-kb *Hin*dIII fragment in the same orientation relative to the original clone (pSTE11-s16) did complement the *ste11* strain. However, because interrupting these two fragments with the 1.7-kb *Hin*dIII fragment insertion (pste11-s12) failed to produce Ste$^+$ transformants, this internal fragment alone was not sufficient for *STE11* function. Therefore, the *STE11* gene spans the *Hin*dIII site that joins the 2.7- and 1.25-kb insert fragments.

The recombinant subclone pste11-s12 was used to construct a partial *ste11* deletion in vitro. This plasmid contained an insertion of the 1.7-kb *Hin*dIII fragment in the *STE11* gene in the same orientation relative to the 2.7-kb insert as that of the original *STE11* clone. Therefore, a *Bam*HI-*Xho*I insert fragment contained a deletion of the 1.25-kb internal *Hin*dIII fragment and was expected to cause sterility when inserted in place of the wild-type allele in the

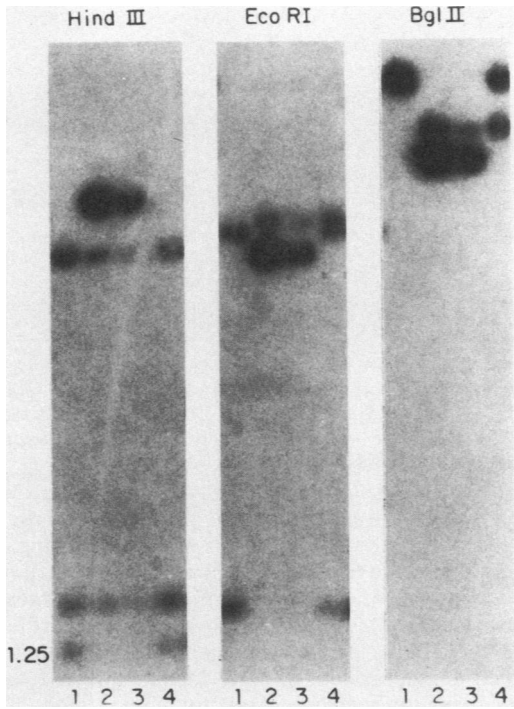

FIG. 2. Blot hybridization analysis of *STE11*$^+$ and *ste11* deletion DNA from strains A2α (*STE11*$^+$) (lanes 1), DC24 (*ste11-Δ1*) (lanes 2), DC25 (*ste11-Δ1*) (lanes 3), and DC26 (*STE11/ste11-Δ1*) (lanes 3). The intensely hybridizing bands correspond to episomal YRp7 DNA. The 1.25-kb *Hin*dIII fragment is indicated.

S. cerevisiae genome. Wild-type haploid strain EG123 was cotransformed with both the *Bam*HI-*Xho*I deletion fragment and YRp7. Because introducing a linear DNA fragment whose ends are homologous to yeast DNA can result in replacement of the recipient sequences with those of the donor (22), it was anticipated that a fraction of the Trp$^+$ transformants would also harbor a 1.25-kb deletion of the *ste11* gene. The Trp$^+$ transformants were recovered and screened for mating competence. Sterile segregants, which arose at a frequency of ca. 0.25%, were retested for their Ste phenotype. The molecular structures of several putative *ste11* deletion strains were assayed by hybridization analysis with pSTE11.3 as the probe (28) (Fig. 2). In strains DC24 and DC25, a simple replacement of the wild-type with the transforming deletion sequences occurred. These strains did not contain the 1.25-kb *Hin*dIII fragment present in wild-type *STE11* cells. Moreover, the single *Bgl*II fragment in strains DC24 and DC25 was 1.25 kb smaller than that observed in the wild type. (The strongly hybridizing bands present in these strains in Fig. 2 but absent in the wild-type parent correspond to the cotransforming YRp7 DNA.) However, one Ste$^-$ transformant, DC26, contained both a wild-type allele and the *ste11* deletion. DC26 had both a wild-type and a mutant *Bgl*II fragment, as well as a normal and mutant *Eco*RI hybridization pattern (Fig. 2). Genetic analysis of this exceptional strain revealed that it was a *MAT***a**/*MAT*α *STE11/ste11-Δ1* diploid. Therefore, its Ste$^-$ phenotype is probably a consequence of heterozygosity at the *MAT* locus rather than of dominance of the *ste11* deletion.

Two partial deletions of the *ste7* gene were similarly constructed from pSTE7.3. The 2.3-kb *Hin*dIII *STE7* fragment contained in this plasmid harbored three *Eco*RV sites (Fig. 1). Thus, it was expected that removal of at least one of the *Eco*RV fragments defined by these sites would create a mutant *ste7* allele. The *ste7* deletion plasmids were generated by religating pSTE7.3 after partial *Eco*RV digestion. Two plasmids that contained a deletion of either the 0.6-kb (*ste7-Δ2*) or 0.53-kb (*ste7-Δ1*) *Eco*RV fragment were recovered. The *ste7-Δ2* deletion was gel purified and recovered as a 1.2-kb *Hin*dIII-*Cla*I fragment, whereas the *ste7-Δ1* allele was isolated as a 1.8-kb *Kpn*I-*Cla*I fragment. Each deletion was introduced into the yeast genome of haploid strains A2α and 80 as described for *ste11-Δ1*. Ste$^-$ transformants were recovered from each putative deletion, indicating that both *Eco*RV fragments are required for *STE7* function. Blot hybridization analysis with pSTE7.3 as the probe (28) was performed to determine the molecular structures of these Ste$^-$ mutants. Two of the mutants, DC60 and DC64, lacked the 0.53- and 0.60-kb *Eco*RV fragments present in the parent strain, respectively (Fig. 3). Identical results were obtained for DC52 and DC56 (data not shown). Additional evidence that these mutants were produced by simple replacement of wild-type with deletion sequences was provided by blot hybridization analyses of *Cla*I digests of wild-type and mutant DNA. In each case, the 3.1-kb and wild-type *Cla*I fragment was truncated by 0.55 kb (DC52 and DC60) or 0.6 kb (DC56 and DC64) in the mutants (data not shown).

Several lines of evidence indicated that the *STE7* and *STE11* genes do not encode essential cellular functions. First, genetic analysis of *ste11-Δ1* and *ste7-Δ1* strains demonstrated that they were all haploid; virtually all of the tetrads analyzed gave rise to four viable spores (data not shown). These data also show that these strains did not carry any recessive lethal suppressors conferred by deletion of an essential gene. Second, the results of blot hybridization analyses of the viable haploid Ste$^-$ derivatives demonstrated that they harbored simple deletions of DNA sequences that were required for *STE* function. No complex rearrangements were detected, nor were cryptic *STE* sequences observed (i.e., duplicate genes, pseudogenes). Third, as described in detail later, these mutants failed to produce a normal *STE* mRNA, indicating that they carried true null mutations.

Identification and expression of *STE7* and *STE11* mRNAs. The mRNA synthesized by the *STE7* gene was identified by hybridization experiments in which poly(A)$^+$-selected RNA from three of the *ste7* deletion strains was probed with

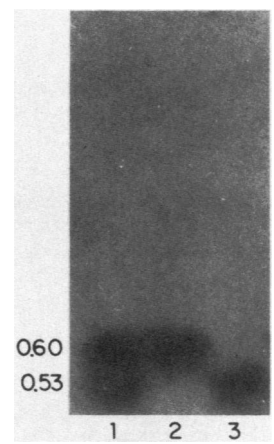

FIG. 3. Blot hybridization analysis of *Eco*RV digests of *STE7*$^+$ and *ste7* deletion DNA derived from strains A2α (*STE7*$^+$) (lane 1), DC60 (*ste7-Δ1*) (lane 2), and DC64 (*ste7-Δ2*) (lane 3). Fragment sizes (in kilobases) are indicated.

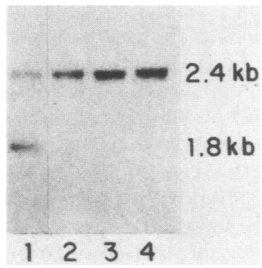

FIG. 4. Northern blot hybridization analysis of RNA isolated from *STE7*$^+$ and *ste7* deletion mutant strains A2α (*STE7*$^+$) (lane 1), DC56 (*ste7-Δ2*) (lane 2), DC60 (*ste7-Δ1*) (lane 3), and DC64 (*ste7-Δ2*) (lane 4). The sizes of hybridizing RNAs are indicated.

^{32}P-labeled *STE7* DNA. The *STE7*$^+$ parent contained two RNA species, 1.8 and 2.4 kb, that hybridized to the pSTE7.3 probe (Fig. 4). However, the absence of the 1.8-kb RNA in all three of the deletion strains identified this RNA as the *STE7* transcript.

The *STE11* mRNA was identified by similar methods. First, two transcripts from *STE*$^+$ cells (1.3 and 2.4 kb) hybridized to a 2.7-kb *Cla*I fragment that contained most, if not all, of the *STE11* gene (see Fig. 1) (Fig. 5). However, only the 2.4-kb RNA shared homology with the 1.25-kb *Hin*dIII fragment that is required for *STE11* function (data not shown), indicating that this RNA was derived from the *STE11* gene. Moreover, this RNA, but not the 1.3-kb species, was absent in the *ste11-Δ1* mutants (Fig. 5), providing further evidence that the 2.4-kb mRNA was the product of the *STE11* gene. In addition to the absence of the 2.4-kb RNA in the deletion mutants was the appearance of a novel 0.71-kb RNA species. This transcript was present only in the mutant and not in the wild-type parent strains and therefore represents the truncated *ste11-Δ1* gene product.

To determine whether expression of the *STE7* and *STE11* genes is transcriptionally controlled by the *MAT* locus, poly(A)$^+$-selected RNA from a variety of haploid and diploid wild-type and *mat* strains (33) was probed with *STE7* and *STE11* sequences (34). Because these genes are required for conjugation in both haploid cell types, their transcripts were expected to be present in both *MAT***a** and *MAT*α cells. However, if these genes only function in mating, then their presence would not be expected in *MAT***a**/*MAT*α diploids, which are mating incompetent. Haploid-specific expression

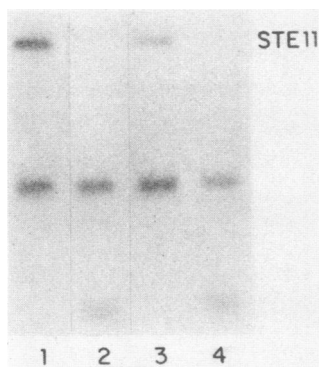

FIG. 5. Northern blot hybridization analysis of RNA isolated from *STE11*$^+$ and *ste11* deletion mutants. Strains: EG123 (*MAT***a** *STE11*$^+$) (lane 1); DC25 (*MAT***a** *ste11-Δ1*) (lane 2); A2α (*MAT*α *STE11*$^+$) (lane 3); DC39 (*MAT*α *ste11-Δ1*) (lane 4).

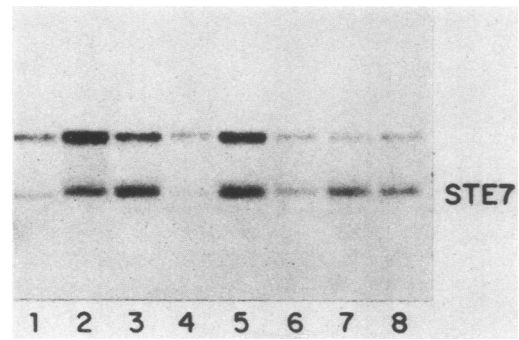

FIG. 6. *STE7*$^+$ RNA levels versus *MAT* genotype. Strains: A2α (*MAT*α) (lane 1); DCX45 (*MAT***a**/*MAT*α) (lane 2); 23ax50 (*mat***a***1-50*) (lane 3); 22 (*matα1-13 MATα2*) (lane 4); 53 (*MATα1 matα2-182*) (lane 5); 12 (*MAT***a**/*MATα1 matα2-182*) (lane 6); 33 (*MAT***a**/*matα1-113 MATα2*) (lane 7); DCX47 (*MAT*α/*mat***a***1-50*) (lane 8).

has been observed for *STE5* (13), a nonspecific sterile gene, and *HO* (7). The 1.8-kb *STE7* mRNA, as well as the 2.4-kb RNA homologous to the pSTE7.3 probe, was present in both *MAT*α haploid and *MAT***a**/*MAT*α diploids (Fig. 6, lanes 1 and 2). These transcripts were also found in the various *mat* mutants that were also tested, demonstrating that *STE7* expression is independent of *MAT* locus control.

Identical results were obtained for *STE11* (Fig. 7). The probe in this experiment, pSTE11.3, contained the entire 5.6-kb cloned insert and hybridized to four mRNA species, one of which was the 2.4-kb *STE11* RNA. Like *STE7*, the *STE11* transcript was synthesized in wild-type haploid (lanes 1 and 2), *mat* mutant haploids (lanes 3–6), the wild type (lane 7), and mutant diploids (lanes 8 and 9). These results demonstrate that *STE11* expression is not subject to transcriptional control by the *MAT* locus.

To verify the validity of the results described above, the *STE11* probe was removed from the filter, and the filter was rehybridized with radioactively labeled *MFα1* DNA. The *MFα1* gene encodes the α cell type mating hormone α-factor (11) and is positively regulated by the *MATα1* gene product (2). The transcriptional profile of *MFα1* RNA was that expected of an α-specific gene (Fig. 8). *MFα1* RNA was

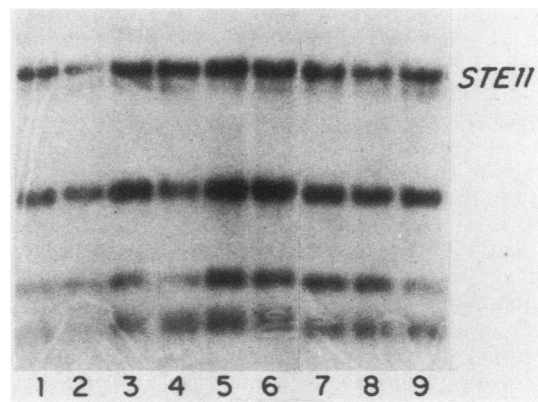

FIG. 7. Effect of *MAT* genotype on *STE11* RNA levels. Strains: EG123 (*MAT***a**) (lane 1); A2α (*MAT*α) (lane 2); 23ax50 (*mat***a***1-50*) (lane 3); 53 (*MATα1 matα2-182*) (lane 4); 27 (*MATα1 matα2-75*) (lane 5); 6 (*matα1-189 matα2-75*) (lane 6); DCX45 (*MAT***a**/*MAT*α) (lane 7); DCX47 (*MAT*α/*mat***a***1-50*); (lane 8); 12 (*MAT***a**/*matα1-182 MATα2*) (lane 9).

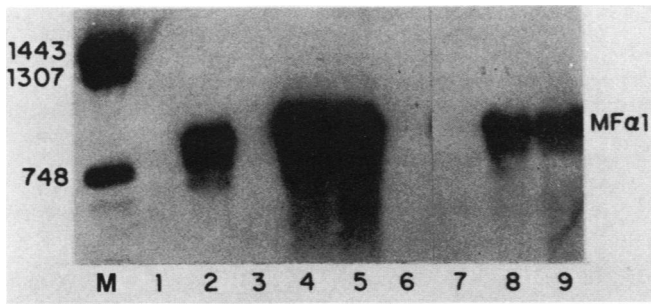

FIG. 8. Effect of *MAT* genotype on *MFα1* RNA levels. Strains: EG123 (*MAT***a**) (lane 1); A2α (*MATα*) (lane 2); 23ax50 (*mat***a***1-50*) (lane 3); 53 (*MATα1 matα2-182*) (lane 4); 27 (*MATα1 matα2-75*) (lane 5); 6 (*matα1-189 matα2-75*) (lane 6); DCX45 (*MAT***a**/*MATα*) (lane 7); DCX47 (*MATα*/*mat***a***1-50*) (lane 8); 12 (*MAT***a**/ mat*α1-182 MATα2*) (lane 9). Lane M, Markers (molecular weight is indicated).

present in *MATα* but not in *MAT***a** haploids or in *MAT***a**/*MATα* diploids. Expression was observed in the *matα2* mutants (lanes 4 and 5), but was abolished by the introduction of a *matα1* mutation (lane 6). That *MFα1* expression exhibited the expected pattern of *MAT* regulation in these RNA preparations demonstrated that the results obtained with the *STE7* and *STE11* probes were not artifactual.

Similar hybridization studies showed that neither *STE7* nor *STE11* controls the transcription of the other (data not shown).

The results of these transcription studies indicated that the *MAT* locus does not have an omnipotent role in cell type determination and processes that depend on cell type expression. Although numerous other α-, **a**-, and nonspecific sterile genes studied to date have been shown to be transcriptionally regulated by *MAT* (2, 7, 10, 13, 20, 21, 27, 29, 31, 36), regulation of three genes (*STE13*, *KEX2*, and *TUP1*) required for α cell function is not (12, 30, 35). Cells carrying mutations in the latter two genes show pleiotropic effects independent of cell type, and the only phenotype ascribed to the *STE13* gene is α-specific sterility (31). The *STE13* gene encodes a membrane-bound dipeptidyl aminopeptidase that is involved in α-factor maturation (8). Because *ste13* mutations cause sterility only in α cells, *STE13* has been classified as an α-specific sterile gene. However, *STE13* expression occurs in all cell types (30). Clearly, such a gene product could act in the proteolytic processing of polypeptides unrelated to cell type expression, obviating the importance of *MAT* locus control. By analogy with the *STE13* example, detection of *STE7* and *STE11* mRNAs in nonmating diploids suggests that the functions these two genes encode may be of a more general nature. If so, it is important to note that they do not encode essential cellular functions. Thus, the effects of *ste7* and *ste11* mutations on the conjugation process may only provide a glimpse into the broader function of these genes.

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